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Ijaz, Usman

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Rust resistance in faba bean (*Vicia faba* L.): Status and strategies for improvement

*[1]Usman Ijaz, [2]Kedar N. Adhikari, [3]Frederick L. Stoddard and [2]Richard Trethowan

[1]Plant Breeding Institute, University of Sydney, Cobbitty, NSW, Australia.

[2]I.A. Watson Grain Research Centre, University of Sydney, Narrabri, NSW, Australia

[3]Department of Food and Environmental Sciences and Viikki Plant Science Centre, University of Helsinki, Finland.

*Corresponding author: usman.ijaz@sydney.edu.au

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Abstract:

Faba bean (*Vicia faba* L.) is an important grain legume used as food and feed. Its production is threatened by abiotic stresses and diseases, of which rust (*Uromyces viciae-fabae*) is one of the major diseases in East and North Africa, China and the northern grain-growing region of Australia. Understanding the genetic and physiological mechanisms of rust resistance in faba bean is in an early phase. The presence of seedling and adult plant resistance genes has been observed. The resistance most frequently utilised in applied plant breeding is race-specific, where the interaction between resistance genes in the host and avirulence genes in the pathogen confers resistance. The main drawback of using race-specific resistance is lack of durability, when deployed singly. Slow rusting or partial resistance, controlled by multiple genes of small effect, is generally non-race specific, so it can be more durable. We present the current knowledge of host resistance and pathogen diversity and propose rational breeding approaches aided with molecular markers to breed durable rust resistance in faba bean.

Key words: Faba bean rust (*Uromyces viciae-fabae*), breeding, major resistance genes, gene pyramiding, molecular markers.

Introduction:

Faba bean (*Vicia faba* L.) is a protein-rich grain legume, grown on all inhabited continents for food and feed, and it is a key source of dietary protein in North and East Africa, the

Middle East and China. It is a diploid (2x=12) species having one of the largest described genomes (13 Gb) among crop legumes, with at least 85% repetitive DNA (Flavell et al. 1974). It is believed to have been domesticated in the Fertile Crescent during the Neolithic era 9000-10000 BP (Tanno and Willcox 2006). Its secondary centres of diversity include the Nile Valley, Central and Eastern Asia, and South America (Duc et al. 2010). Faba bean is the sixth most important grain legume with 4.3 million tonnes annual production worldwide in 2014 (FAOSTAT 2017). Australia is the fourth largest producer (after China, Ethiopia and the UK) and the largest exporter globally (Eurostat 2016; FAOSTAT 2017), with nearly 85% of its total produce going to Egypt (70%) and Saudi Arabia (15%) (ABSTAT 2015). Similar to other legumes, faba bean is a good source of renewable nitrogen through biological nitrogen fixation (Jensen et al. 2010). It has a mixed breeding system, averaging one-third outcrossing and two-thirds inbreeding, which adds complexity to populations in the field, their genetic and phenotypic analysis, and practical plant breeding (Stoddard and Bond 1987).

Faba bean area decreased worldwide from 1962 to 1991, and since then has more or less stabilised at 2.1-2.7 million hectares, mostly across Asia and the Mediterranean region FAOSTAT 2017). Apart from the commodity price, one of the reasons for decline is the prevalence of pathogenic fungi and the absence of disease-resistant cultivars (Sillero et al. 2010). Measures to control diseases include chemicals, biological control agents and crop management or cultural practices (Sillero et al. 2010). Systemic fungicides provide rapid and effective control, but they are expensive and can be detrimental to the environment (Emeran et al. 2011). Pathogens can also develop insensitivity following prolonged exposure to chemicals (Crute 1992). Biological control agents for pathogenic fungi are not yet available commercially (Stoddard et al. 2010). Therefore, breeding cultivars for resistance is widely considered the most efficient, cost effective and environmentally safe method of disease control (Sillero et al. 2010).

Rust is a major disease of faba bean in North Africa and the northern parts of the Australian grain belt (northern New South Wales and southern Queensland). The disease is caused by the biotrophic fungus *Uromyces viciae-fabae* (Pers.) J. Schrot., a macrocyclic rust and true obligate parasite with a sexual phase that does not require an alternate host to complete its lifecycle (Mendgen 1997; Voegele 2006). In addition to faba bean, it can parasitize common vetch (*Vicia sativa* L.), pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medik.) (Conner and Bernier 1982a; Cummins 1978). Rust usually occurs at the time of pod filling in Europe

and southern Australia, causing up to 20% yield reduction (Sillero et al. 2011), although early rust epidemics can cause up to 70% loss of the standing crop (Liang 1986; Rashid and Bernier 1991). Losses up to 30% were estimated in northern New South Wales where the disease occurs in the seedling stage. Rust resistance in general restricts fungal proliferation before sporulation. In contrast to other pathosystems such as soybean-rust (*Phakopsora pachyrhizi* Syd.), for which complete monogenic resistance exists (Silva et al. 2008), resistance in faba bean is generally described as incomplete (Garcia et al. 2008; Miklas et al. 2006). Incomplete resistance, a type of quantitative resistance either expressed as hypersensitive type (Adhikari et al. 2016a; Sillero et al. 2000) with visible necrosis/chlorosis or non-hypersensitive type (Herath et al. 2001; Polignano et al. 1990; Rashid and Bernier 1984; 1991; Sillero et al. 2000) typically fits into the definition of partial resistance or field resistance (Parlevliet 1983). The hypersensitive reaction type, causing significant restriction on fungal growth and reduction of an infection type, is still considered as incomplete in faba bean (Sillero et al. 2010).

In this review, we highlight the current state of knowledge of rust resistance in faba bean and explore options for extending the genetic diversity of resistance and enhancing the resistance of this crop with new and evolving genomic technologies.

A brief history of rust resistance

The genetic control of plant disease resistance was established soon after the rediscovery of Mendel's work, when Biffen (1905) reported that resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici)* of common wheat (*Triticum aestivum* L. emend Thell.) was controlled by a major gene with partial dominance. Variation in a pathogen population, resulting in variability for virulence (Barrus 1911) and resistance (McRostie 1919), was noted soon thereafter. Consequently, the concept of physiological races was defined. Physiological races are morphologically indistinguishable, but they can be differentiated in their reaction against a set of host lines known as differentials.

Studies on the inheritance of host resistance and resistance breakdown based on hypersensitive responses culminated in Flor's (1956) gene-for-gene hypothesis, wherein genes for host resistance (*R*) and pathogen avirulence (*avr*) control the outcome of the host-pathogen interaction. Virulent mutated pathotypes do not elicit the hypersensitive response because the corresponding *R* gene is no longer effective. Mutation events resulting in a deletion or substitution of an essential amino acid responsible for protein function may cause

a complete loss of the *avr* gene (de Wit et al. 2009). Thus, genetic resistance in obligate parasites such as rust can be highly race-specific (Keller et al. 2000).

Single *R* genes have generally failed to provide long-lasting resistance to rust (Johnson and Law 1973), because new virulent pathotypes frequently evolve, forcing breeders to seek new resistance genes. Lack of durability of resistance is not common to all plant pathosystems, but it is common among air-borne pathogens that possess both sexual and asexual cycles of reproduction. In the latter system, breeding for disease resistance became locked in a 'boom-bust' cycle (Pink 2002), where resistant cultivars are sown on large areas, exerting considerable selection pressure on avirulence in the pathogen. This sequence led to the concept of multi genes and gene pyramiding, discussed later.

Genetic diversity of rust resistance in faba bean

The success of resistance breeding depends upon the presence of genetic variability and our ability to detect and utilise it. This variability is captured in the collection of germplasm. The largest collection of faba bean worldwide is maintained by ICARDA, Terbol, Lebanon,with over ten thousand accessions from 71 countries. The second largest reserve with 5,200 accessions is preserved at the Chinese Academy of Agriculture Research, Beijing, China followed by 2,445 collections in the Australian Grains Genebank, Victoria, Australia. The USA, Russia, Ethiopia, France, Spain, Germany and Italy also have large numbers of accessions.

No wild species is sufficiently closely related to faba bean that fertile hybrids have been derived, so genetic diversity is restricted to the primary gene pool of the species itself (Cubero 2011). Accessions from the Mediterranean region are considered to have the best potential to provide genetic variation for rust resistance (Maalouf et al. 2010). Genotypes showing significant rust resistance with late necrosis originated from Central Europe (V-300, V-313, V-1271, V-1272), East Africa (2N-34) and Spain (V-1335) (Sillero et al. 2000). Most of these reported rust resistance germplasm lines were already imported in Australia and named with Australian accession numbers located at Australian Grain Genebank, Horsham, Victoria (Table 1). However, interaction of these resistance genes with prevailing faba bean rust pathotypes is unknown. Interspecific sources of resistance cannot be transferred to faba bean by conventional breeding methods, and the practical significance of induced mutation is relatively low due to low success rates (van Harten 1998). The species is generally intractable to tissue culture, but successful transformation by Agrobacterium has

been reported (Hanafy et al. 2013), opening the door for eventual interspecific transfer of resistance or editing of endogenous genes.

Pioneering work on rust resistance in faba bean was done in Canada in the 1980s, followed by Spain and Australia in recent years (Marcellos et al. 1995; Rose and van Leur 2006), with the identification of new genes and preservation of pathogen isolates (Adhikari et al. 2016a). Three race-specific genes (*fr1*, *fr2* and *fr3*) were found for rust resistance in seven faba bean inbred lines in Canada (Conner and Bernier 1982b). Conner and Bernier (1982c) identified seven genes conferring resistance to the specific rust isolates of Manitoba, Canada. Four years later, the same group of scientists reported the presence of five genes conditioning resistance against rust isolates collected from Canada (Rashid and Bernier 1986a). The hypersensitive resistance response has been mapped to gene *Uvf-1* by a group based in Spain (Avila et al. 2003).

The national faba bean breeding programme in Australia aims to provide cultivars for southern regions (South Australia and Victoria) and the northern plains (New South Wales and Queensland). Rust is a major disease in the northern plains, so breeding for its resistance is one of the main objectives. A great deal of genetic diversity of rust resistance at the seedling stage has been found in the Australian collection of faba bean germplasm (Fig. 1). The first moderately rust resistant cultivar, Doza, was released for cultivation in the northern plains in 2008, followed by PBA Warda and PBA Nasma during 2012 and 2016, respectively (Pulse Australia 2017). Three rust resistance genes were reported in segregating populations from crosses of exotic (Ac1227#14908 and Ac1655) accessions and local genotype Doza#12034 (Ijaz and Adhikari 2016a) (Fig. 1). Two of these genes (from Ac1655 and Doza#12034) were independently dominant, along with possible additive response (Adhikari et al. 2016a), and were mapped onto two different chromosomes (Sudhesh et al. 2016). However, the resistance in Ac1227#14908 was governed by complimentary gene action (Ijaz and Adhikari 2016b).

The nomenclature for rust-resistance genes in faba bean is not uniform and coordinated. The Canadians named their genes *fr*, whereas the Spanish named theirs *Uvf*, so a universal system of naming the resistance genes and pathogen variants (pathotypes) is needed similar to that used for wheat (McIntosh et al. 1995). This will enable comparison and verification of different genes identified in different areas.

Slow rusting: an insight into faba bean breeding

159 Several sources of slow rusting in faba bean have been reported (Conner and Bernier 1982d,
160 Torres et al. 2006). Slow rusting limits the development and spread of infection, thus
161 allowing the plant to outgrow the disease with little impact on productivity. Slow rusting or
162 partial rust resistance has been reported in many crops, such as maize and sweet corn (*Zea*
163 *mays* L.) (Hooker 1969; Groth et al. 1983), wheat (Singh et al. 2000) and barley (*Hordeum*
164 *vulgare* L.) (Wilcoxson 1981). Slow rusting against *U. viciae-fabae* has also been identified
165 in pea (*Pisum sativum* L.) (Chand et al. 2006) and lentil (Gupta and Singh 2010).

166 Generally, slow rusting is considered as durable because of its stable expression and reduced
167 selection pressure on the pathogen (Bond et al. 1994). Macroscopically, slow rusting in faba
168 bean is characterized by reduced lesion size and infection frequency with a longer latent
169 period (Sillero et al. 2000). Microscopy revealed that reduced hyphal growth inhibited
170 haustorial maturation, thereby limiting the formation of sporulating colonies (Rubiales and
171 Sillero 2003; Sillero and Rubiales 2002).

172 Reduced yield losses (1-2%) were reported in a slow-rusting population compared to a
173 susceptible population (61%) (Rashid et al. 1991) in faba bean. The area under the disease
174 progress curve (AUDPC) provides a way to estimate slow rusting, and significant diversity
175 in this trait has been reported in faba bean (Rashid and Bernier 1986b), lentil (Negussie et
176 al. 2005) and pea (Singh et al. 2015). Low AUDPC in some accessions may not be entirely
177 due to slow rusting because other factors, including cell wall thickness, number of stomata
178 (Heath et al. 1990), temperature, leaf wetness and light (Joseph and Hering 1997) influence
179 the disease development.

180 Information on the genetic basis of slow rusting in faba bean is limited. Both cytoplasmic
181 and nuclear genes have been implicated (Stoddard and Herath 2001). The non-
182 hypersensitive reaction is believed to be polygenic with minor effects, but such minor genes
183 have not been identified or characterized. Torres et al. (2006) proposed studying this type of
184 resistance using mapping populations and quantitative analysis, but no results have yet come
185 to light.

186 **Understanding the pathogen: *Uromyces viciae-fabae***

187 *Uromyces*, with about 600 species, is the second most important genus after *Puccinia* in the
188 order Pucciniales (Maier et al. 2003). *Puccinia* species mainly infect grasses while
189 *Uromyces* infects legumes. Genome sequence information is available for *Puccinia*
190 *striiformis* f. sp. *tritici* (*Pst*) and *Puccinia graminis* f. sp. *tritici* (*Pgt*) (Cantu et al. 2013;

Duplessis et al. 2011; Zheng et al. 2013). Research activities have been concentrated on a very limited number of the 7,000 known rust fungi. Despite their ecological and economic values, little is known about the phylogeny within and between these groups. On the basis of sequences of the beta-tubulin 1 gene, a phylogeny showed that neither *Puccinia* nor *Uromyces* had evolved as a monophyletic group, and species of both fell into two distinct clades (Van der Merwe et al. 2007). Moreover, it was speculated that the pathogen genera diversified in parallel with their respective hosts under the situation when unrelated hosts met randomly in close proximity due to similar ecological needs.

Faba bean rust is an autoicous and macrocyclic fungus exhibiting completes all five spores stages, telio-, pycnio- basidio-, aecio- and urediospores, in a single host (Mendgen and Deising 1993). Host-specialized isolates were characterized on the basis of distinct spore dimension and infection structure morphology (Emeran et al. 2005), suggesting that *U. viciae-fabae* may be divided into intraspecific groups with differential pathogenicity to these hosts (Rubiales et al. 2013). This intraspecific distribution was confirmed through RAPD DNA markers (Emeran et al. 2008). Therefore, *Uromyces viciae-fabae sensu lato* is apparently a species complex that cannot be divided into *formae speciales*.

In addition to the knowledge of host diversity, understanding the pathogen is also a crucial step to ensure longevity of resistance. As faba bean rust is an obligate parasite with a sexual phase, new and virulent pathotypes can evolve frequently, thus requiring continuous genetic improvement to ensure that new cultivars are resistant to prevailing pathotypes. There is little evidence of faba bean rust pathogen diversity in Australia. Herath (2000) found that some isolates were generally less virulent than others, and showed some isozyme variation among these isolates, but further advances have been held back by the lack of differential hosts to characterise the various isolates, despite the early progress in Canada in this regard. It is essential that pathogen diversity be properly understood, including the implications for virulence, but the differentials developed in Spain (Avila et al. 2003) and Canada (Conner and Bernier 1982b) have not been used elsewhere, making it difficult to judge the effectiveness of one gene in other areas. Nine faba bean rust races were characterised from twelve faba bean lines on the basis of differential pathogenicity in Australia (Ijaz et al. 2017). Moreover, a binary nomenclature system (Steadman et al. 2002) for naming pathotypes is under development and will be available soon. These pathotypes are cryopreserved at the University of Sydney's Plant Breeding Institute, Cobbitty, Australia.

Link et al. (2014) provided an early insight into the genomic architecture of *U. viciae-fabae*. Sequencing was performed by using HiSeq2000 with a 500 nt library. Three different estimates of genome size were made: with 15-mer analysis, genome size was estimated at 329 Mb, whereas 17-mer analysis predicted a genome size of 422 Mb and filtered assembly estimated 216 Mb. Flow cytometry on the same isolate generated a size of 379 Mb (Tavares et al. 2014). Thus, the estimate of the actual genome size is in the range of 330 and 422 Mb, making it about 4 times the size of *P. graminis* f. sp. *tritici*, indicating a high significance of transposable elements (TE) (Duplessis et al. 2011). Link et al. (2014) identified numerous families of secreted proteins and candidate effectors, and reported 62 genes encoding proteins secreted from haustoria that have presumed roles in infection and specificity of *U. viciae-fabae*.

Deployment of resistance genes with major effects

Disease resistance is generally inherited as a dominant factor in natural populations (Barrett 1985), as natural selection acts on it favourably. Resistance based on major hypersensitive genes is usually considered non-durable (Stuthman et al. 2007) according to the gene-for-gene hypothesis. Hypersensitivity has been observed among faba bean genotypes, where necrosis appears during the later stages of rust proliferation and restricts fungal growth by controlled cell death (Sillero et al. 2000; Torres et al. 2006). Hypersensitivity is easily detectable with specific pathotypes (Emeran et al. 2005; Rojas Molina et al. 2006). Avila et al. (2003) and Adhikari et al. (2016b) showed that this type of hypersensitive response in faba bean is under the control of major genes. Nevertheless, the longevity of major genes in faba bean is less understood than in wheat as there is limited knowledge on pathogen variability. If these resistance genes of major effect can be deployed in combination through gene pyramiding, multiline cultivars or gene mixtures, the durability of resistance can be increased.

a. Pyramiding resistance genes

Gene pyramiding is the simultaneous deployment of more than one *R* gene in the same cultivar. Such *R* gene pyramids can provide long-lasting resistance because multiple simultaneous mutational events in *avr* genes are required to elicit susceptibility in the host. A particular gene combination (*Avr*/*R*) will remain effective as long as matching virulence is not widespread in the population, but it is difficult to identify individual *R* genes in a

complex pyramid unless virulent pathotypes or specific DNA markers are available (Pink 2002).

Gene pyramiding provides an opportunity to achieve a 'clean crop' and ensures crop uniformity because all genes are deployed in a uniform genetic background. Nevertheless, R gene pyramids are vulnerable to virulence if the individual components of resistance are deployed singly in other cultivars grown in the same region (Parlevliet 1997). The R gene in a pyramid exerts a strong unidirectional selection pressure against the matching virulence in a pathogen population. Therefore, pathogen monitoring to identify new virulent pathotypes is essential to minimise risk through early warning. The number of R genes in a pyramid should determine the spread of emerging virulent pathotypes in a pathogen population (Kolmer 1992; Mundt 1991), but conflicting results have been reported. For example, Mundt (1991) reported that an R gene pyramid failed to provide resistance against stem rust in wheat. This breakdown of resistance started a debate over the value of R-gene pyramids. Although there are some examples of cultivars where the R gene pyramid ensured durability of resistance, there is no clear association between the number of R genes in a pyramid and durability. The successful use of gene pyramiding against rust in faba bean has not been reported, but two recently reported (Adhikari et al. 2016a) seedling rust resistance genes provide a potential for pyramiding because the combination of both genes prevented fungal sporulation (Ijaz et al. Unpubl. data) (Fig.1). This kind of very strong resistant infection type has not been witnessed before in faba bean.

b. Using multilines and crop mixtures

In comparison to domestic crops, wild species are more heterogeneous for resistance genes and thus avoid disease epidemics (Bevan et al. 1993; Okamura and Ouchi 2007). The most appropriate breeding strategies that utilize crop diversity are multiline development (Browning and Frey 1969) and cultivar mixtures (Wolfe and Barrett 1980). A multiline is a composite of agronomically similar genotypes that differ for a few traits, such as resistance to different pathogen races (Jensen 1952). Wolfe (1985) defined a cultivar mixture as a "mixture of cultivars that vary for many characters including disease resistance, but have sufficient similarities to be grown together".

Despite the documented benefits of growing cultivar mixtures over pure stands (Wolfe and Finckh 1997), their commercial impact has been low. Lack of uniformity can influence seed quality and certification, leading to legislative constraints on seed production and

distribution (Wolfe et al. 1992). Farmers often oppose growing mixtures because of uneven crop appearance. There are examples of large scale utilization of mixtures in barley (Wolfe and McDermott 1994), wheat (Garrett and Mundt 1999; Wolfe 1985) and rice (Skamnioti and Gurr 2009; Zhu et al. 2000), but their recent use for disease management is limited. Resistance to rust has never been deployed in mixtures or multilines in faba bean, largely because resistance is poorly characterized in this crop, but there is no reason to believe that multilines or mixtures will not work, as our understanding of genetic diversity for resistance increases. It is important to initiate investigation by testing a mixture of rust-susceptible and -resistant faba bean genotypes for disease suppression and yield stability.

Using systemically acquired resistance (SAR)

Systemic acquired resistance (SAR) is a "whole plant" resistance response that is usually triggered under biotic and abiotic stresses (Walters and Murray 1992). SAR is accompanied by the up-regulation of large numbers of so-called pathogenesis-related (PR) genes (Melchers and Stuiver 2000) that have an important role in defence against fungal infection and genes encoding fungitoxic or fungistatic proteins provide resistance against invading fungi (Cornelissen and Melchers 1993). Murray and Walters (1992) reported that inoculation of lower leaves of faba bean with rust spores increased resistance in the upper leaves and enhanced their photosynthesis, as shown by the significantly increased accumulation of ^{14}C-labelled assimilate after $^{14}CO_2$ was fed to the upper leaves. However, the virulence of the inoculated rust pathotype was unknown. Systemic resistance in faba bean can be induced by exposure of plants to certain chemicals. Application of salicylic acid or benzo-(1,2,3)-thiadiazole-7-carbothionic acid (benzothiadiazole, BTH) increased the resistance of faba bean leaves to rust, Ascochyta blight (*Ascochyta fabae* Speg.) and broomrape (*Orobanche crenata* Forsk.) (Sillero et al. 2012). Similarly, foliar application of BTH and DL-β-aminobutyric acid (BABA) to pea significantly reduced pea rust (*Uromyces pisi*) infection levels locally and systemically (Barilli et al. 2010). Cheng et al. (2012) characterized the resistance mechanism in faba bean against *Puccinia striiformis* f. sp. *tritici* (*Pst*) as non-host. Although no symptoms were visible on inoculated leaves, microscopy showed that the fungus successfully located the stomata, but failed to develop mature haustoria. Transcriptomic expression revealed the up-regulation of seven defence-related genes during *Pst* infection (Table 2). In the light of these observations, and the strong similarities in mechanisms of resistance to adapted and unadapted pathogens, SAR in faba bean may be

318 worth studying in greater detail. When the PR genes are validated, they can be used against
319 rust.

320 **Molecular breeding technologies**

321 Early genetic studies in crops were based on morphological, cytological and biochemical
322 evaluation (Xu 2010). A sequence of DNA markers, from Restriction Fragment Length
323 Polymorphism (RFLP), through Amplified Fragment Length Polymorphism (AFLP),
324 Random Amplified Polymorphic DNA (RAPD), and Simple Sequence Repeats (SSR) to
325 Single Nucleotide Polymorphisms (SNP) has allowed increasingly high resolution and
326 closer linkage to the traits of interest, which can assist gene tracking during selection and
327 breeding (Collard et al. 2005). Markers that are closely linked (less than 5 cM of genetic
328 distance) with the gene of interest reduce the probability of recombination and can provide
329 reliable selection in crop improvement (Jiang 2013). Use of flanking markers increases the
330 probability of success because of the low likelihood of double crossovers in a short length
331 of chromosome. MAS is a practical and effective way to pyramid rust resistance genes if the
332 genes and closely linked markers are available (Servin et al. 2004) and is already in use for
333 other traits such as vicine-convicine content (Khazaei et al. 2015). Association mapping and
334 linkage mapping are powerful tools used for identifying QTLs and genes responsible for a
335 particular phenotype (Collard and Mackill 2008). To date, only one of the successful
336 mapping efforts in faba bean is relevant to rust resistance (Avila et al. 2003), where bulk
337 segregant analysis (BSA) was done to tag RAPD markers linked to the hypersensitive
338 response in faba bean line 2N-52 against rust race 1 in Spain. Three RAPD markers, namely
339 $OPD13_{736}$, $OPL18_{1032}$ and $OPI20_{900}$, were mapped in coupling phase to the resistance gene
340 *Uvf-1*. Two additional markers were linked to the gene in repulsion at distances of 9.9 cM
341 ($OPP02_{1171}$) and 11.5 cM ($OPR07_{930}$). The line 2N-52 used in this study was resistant to
342 seven other rust isolates (2, 4, 5, 8, 9, 10 and 13) (Emeran et al. 2001), but this broad
343 resistance was not solely attributable to gene *Uvf*-1.

344 Recent advances in the next generation sequencing (NGS) technologies provide a platform
345 to sequence a large volume of individuals efficiently and cost-effectively (Morozova and
346 Marra 2008). This advancement opens a new era in agricultural research including the
347 identification of key genes correlated with breeding traits through SNPs and Genome-Wide
348 Association Analysis Studies (GWAS) (Lam et al. 2010). Another utility of sequence data
349 is to identify flanking SSR regions to develop locus-specific markers for downstream
350 genotyping. In faba bean, the first major contribution was the release of 5000 Expressed

Sequence Tags (EST) from the early to mid-developing embryo of broad bean cultivar 'Windsor' (Ray and Georges 2010). Yang et al. (2012) constructed a library of 125,559 putative SSR sequences and determined a set of 28,503 primers, of which 15,094 primers showed polymorphism among 32 diverse faba bean accessions. The observed heterozygosity ranged from 0.0908 to 0.840. The validation of these markers based on Nei's genetic distance showed their high quality and effectiveness. Kaur et al. (2014b) undertook 454 sequencing of the transcriptome to underpin SSR to significantly increase the volume of transcriptome data in mixed genotypes. In addition, Kaur et al. (2014a) evaluated inter- and intra-population diversity in 45 diverse accessions of faba bean by using a set of 768 genome-wide SNP markers, of which 657 were successfully amplified. The genetic structure of these accessions was analysed through a neighbour-joining (NJ) dendrogram that categorized them into two major groups and several sub-groups. Group I was comprised of accessions from Ecuador, Australia and ICARDA, Group IIA consisted of African and European materials, whereas Groups IIB and IIC were predominantly of Australian origin. These results confirmed that a high level of heterozygosity is present, presumably due to the partial allogamous nature of the species. Subsequently, transcriptome studies were focused on multiple inbred lines (Ocaña et al. 2015) as well as multiple tissues (Ray et al. 2015). The deepest transcriptome coverage yet produced through Illumina sequencing library of mixed tissues from cultivar 'Fiord' (Arun-Chinnappa and McCurdy 2015; Zhang et al. 2015), which was the first released cultivar in Australia and was highly susceptible to all known variants of rust. These available transcript datasets can be used to compare with transcriptomes of resistant lines to identify transcripts conferring resistance. Webb et al. (2016) developed and validated 845 SNPs by using NGS. Each SNP was assigned by BLAST analysis to a single *Medicago* orthologue and a set of 757 markers was used to genotype six mapping populations. The resulting linkage maps were merged into a single consensus map of 687 SNPs on six linkage groups, each corresponding to one chromosome. Substantial collinearity was found with the fully sequenced genome of barrel medic (*Medicago truncatula* L.), allowing relatively easy prediction of gene contents. Recently, using Illumina paired end sequencing, genomic reads were assembled *sub de novo* in to contigs of size 50-5000 bp, out of which, over 85% sequences did not align with known gene, representing ~10% known repetitive genetic elements (Cooper et al. 2017).

At present, significant transcriptome datasets are available in the arsenal of the faba bean breeders. These recent advances in faba bean genomics provide background for successful

manipulation of genomics and proteomics tools. When rust response phenotypes are linked with genotypes at a high level of resolution, resistance breeding will accelerate through the high selection efficiency that can then be used in pyramiding genes and constructing multilines or mixtures.

Conclusion

Knowledge is growing about host-pathogen interactions, the diversity of host resistance genes and their genetic bases, and pathogen variation of faba bean rust. The resistance mechanisms in faba bean germplasm are well established as either slow-rusting or hypersensitive responses. Closely linked molecular markers for both types of resistance are needed for the successful development of durable cultivars. Although the progenitor of faba bean has not yet been identified, enough variation for rust resistance seems to be present in the cultivated species. Although *Uromyces* is the second most important rust genus after *Puccinia*, its physiology and genetics have received little attention.

Gene pyramiding is a possible avenue for deploying resistance genes, but needs to be underpinned by an extensive search for new rust resistance sources and the concurrent identification of genes and linked molecular markers. With current mapping efforts on known seedling genes for rust resistance, marker-assisted gene pyramiding can be considered as a viable strategy in resistance breeding. However, continuous monitoring will be needed to keep an eye on the evolution of virulent pathotypes against these effective resistance genes. As the virulence evolves, the pyramid will have to be reconstructed with new resistance sources to avoid epidemics. Although mixtures or multilines can provide some measure of resistance, their successful commercialisation requires that they should provide more benefits to farmers than provided by conventional cultivars. Until now, whole plant resistance or antifungal gene strategy is unable to provide a strong successful reference in applied breeding, but with growing understanding, it has potential in the future. Moreover, genes of systemically acquired resistance against a non-host pathogen have been well studied in faba bean, and have potential for use against host pathogens such as rust.

Genomics and biotechnology have been little used in breeding faba bean for rust resistance because of it being a minor crop with a large genome size, and the resistance is complex in nature. Genetic and genomic knowledge has advanced far enough now to assist in the identification of genes and their successful deployment in this crop, and we can expect soon to have plenty of genome-wide SNP variants for rust resistance across diverse backgrounds.

In parallel, these datasets can also be used for gene mapping, gene cloning and successful gene transformation. We can anticipate that plant breeders will be able to use genomics to identify the resistance with high accuracy, to ensure a rust-free crop in the future.

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875

876 Table 1 Reported rust resistant germplasm resources in Australia.

Australian accession No.	Donor Accession No.	Source	Country	Reference
AC341	ILB53	ICARDA	England	Rashid and Bernier (1984)
AC408	ILB159	ICARDA	Greece	Rashid and Bernier (1984)
AC466	BPL7	ICARDA	Jordan	Rashid and Bernier (1984)
AC472	ILB226	ICARDA	Turkey	Bond et al. (1994)
AC479	BPL8	ICARDA	Jordan	Rashid and Bernier (1984)
AC481	BPL 260	ICARDA	Greece	Bond et al. (1994)
AC547	ILB318	ICARDA	England	Bond et al. (1994)
AC628	BPL8	ICARDA	Jordan	Rashid and Bernier (1984)
AC630	ILB403	ICARDA	Tunisia	Bond et al. (1994)
AC631	BPL260	ICARDA	Greece	Rashid and Bernier (1984)
AC638	ILB411	ICARDA	England	Bond et al. (1994)
AC647	ILB420	ICARDA	Iran	Rashid and Bernier (1984)
AC658	ILB431	ICARDA	Algeria	Rashid and Bernier (1984)
AC832	BPL 309	ICARDA	Turkey	Bond et al. (1994)
AC848	BPL406	ICARDA	Spain	Bond et al. (1994)
AC849	BPL417	ICARDA	Spain	Bond et al. (1994)
AC858	BPL484	ICARDA	Uruguay	Bond et al. (1994)
AC859	BPL427	ICARDA	Spain	Bond et al. (1994)
AC860	BPL533	ICARDA	USA	Bond et al. (1994)
AC983	BPL490	ICARDA	Spain	Bond et al. (1994)
AC984	BPL524	ICARDA	Japan	Bond et al. (1994)
AC999	BPL539	ICARDA	China	Bond et al. (1994)
AC1044	BPL261	ICARDA	Greece	Bond et al. (1994)
AC1045	BPL263	ICARDA	Greece	Bond et al. (1994)
AC1269	BPL 710	ICARDA	Ecuador	Bond et al. (1994)
AC1272	BPL 1179	ICARDA	Ecuador	Bond et al. (1994)

AC1443	BPL 627	ICARDA	Spain	Bond et al. (1994)
AC1652	2N-34	Spanish germplasm	Spain	Bernier and Conner (1982)
AC1653	2N-52	Spanish germplasm	Spain	Bernier and Conner (1982)
AC1654	VF-176	Spanish germplasm	Spain	Sillero et al. (2000)
AC1655	V-300	Spanish germplasm	Spain	Sillero et al. (2000)
AC1656	V-1271	Spanish germplasm	Spain	Sillero et al. (2000)
AC1657	V-1272	Spanish germplasm	Spain	Sillero et al. (2000)
AC1658	V-1273	Spanish germplasm	Spain	Sillero et al. (2000)

877

878 Table 2 Reported defence-related candidate genes in faba bean (Cheng et al. 2012)

Gene	Description	Related species	Arabidopsis homolog (TAIR BLAST)
	Basal resistance		
PR1	Pathogenesis-related gene 1	*Pisum sativum*	AT2G14610.1
PR2	Beta-1,3 endoglucanase	*Pisum sativum*	AT3G57260.1
PR5	Thaumatin like protein	*Medicago truncatula*	-
PR10	Pathogenesis-related gene 10	*Pisum sativum*	No sequence
	Oxidative stress responses		
SOS	Superoxide dismutase	*Pisum sativum*	AT5G18100.1
CAT	Catalase	*Pisum sativum*	AT1G20620.1
	Papillary callose formation		
GSL5	Glucan synthase-like 5	*Medicago truncatula*	AT4G03550.1

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Fig. 1 Characterisation of three seedling resistance types in faba bean germplasm. AC1227#14908 gave higher reaction type (complementary gene), Ac1655 produced small pustules with necrotic lesions, Doza#12034 is showing aggressive chlorosis leading to leaf death, Ac1655 × Doza#12034 hybrid (F_1) completely inhibited fungal sporulation and Fiord showed large sporulating pustules without necrosis or chlorosis against isolate Uvf-8.